REMARKS

Support for claim 31 can be found throughout the specification, e.g., on Page 4, line 27-Page 5, line 2; Page 8, lines 10-14; Page 9, lines 1-22. Support for claim 32 can be found in the specification, e.g., Page 4, line 27-Page 5, line 2; Page 27, lines 1-15.

Rejections under §101 and §112, first paragraph

The specification describes numerous credible, specific, and substantial utilities for the claimed polypeptides, including the use of antibodies for immunological detection of epididymis tissue (e.g., Page 12, line 20-Page 13, line 5) and the use of antibodies (and other ligands) for contraception purposes (e.g., Page 14, line 28-Page 15, line 10).

A specification which contains a disclosure of utility which corresponds in scope to the subject matter sought to be patented must be taken as sufficient to satisfy the utility requirement of § 101 for the entire claimed subject matter unless there is a reason for one skilled in the art to question the objective truth of the statement of utility or its scope. See, e.g., *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995); MPEP §2107.02.

The contraception utility has been rejected, allegedly "Because the instant specification has failed to credibly identify a physiological process which has been shown to be influenced by the activation or inhibition of a putative receptor protein of the instant invention an artisan would have no way of predicting what effects the administration of that ligand to an organism would have. If one can not predict the effect that the administration of a ligand of the putative receptor of the instant invention is going to have on an organism then it is unclear as to what practical benefit is derived by the public from the identification of that ligand."

The examiner has not provided any scientific reason to doubt the claimed contraceptive utility. The only objection raised to it is that the specification has not disclosed a mechanism to explain the contraceptive effect. However, there is no requirement to explain how an invention

works. Knowledge of mechanism is at most only relevant where the disclosed utility is not believable. See, e.g., *Newman v. Quigg*, 877 F.2d 1575, 11 USPQ2d 1340 (Fed. Cir. 1989) having claims to a perpetual motion machine (i.e., energy output is greater than energy required to operate the machine); *In re Dash and Keefe* (Fed. Cir. 2004; no-precedential) having claims to a cold fusion machine. The examiner has not explained why it is inherently incredible to believe, e.g., that ligands to HE6, such as antibodies, as disclosed in the pending application would act as contraceptives in the manner disclosed in the specification. As shown in the attached publication by O'Rand et al., Science, 306:1189, 2004 (Exhibit 1), male immunocontraception was demonstrated to be effective in a primate animal model, and is therefore an established practical utility.

Although unnecessary, since the examiner has failed to establish a *prima facie* case of lack of utility, attached is a publication (Exhibit 2) by Gottwald et al. Dr. Gottwald is an employee of the assignee of the present application and previously provided a Declaration under §1.132. This article describes the efforts to identify male contraceptive agents. Evidence is provided of the specificity of HE6 for epididymis (e.g., Gottwald et al., Fig. 2) and its importance in male fertility (e.g., Gottwald et al., Pages 6-7) as described as in the specification as filed. For example, HE6 knockout mouse were almost sterile. See, Paragraph spanning Pages 6-7 of Gottwald et al. See, also previously filed Declaration by Gottwald under §1.132. On Pages 2-3 of the publication, the authors describe different criteria for identifying targets for male contraception agents. HE6 is evaluated by these criteria, and the authors conclude (e.g., on Page 8, second column) that it is a valid target for contraception purposes. Thus, the stated contraception utility in the application is clearly credible to the person of ordinary skill in the art, the standard set forth in the Patent Office's Guidelines. See, MPEP §2107. II. Examination Guidelines for the Utility Requirement.

It is not necessary to provide evidence that a particular compound (e.g., an antibody to HE6) was used in a therapeutic context. As discussed in *In re Brana*, this is not the correct

standard to apply. "Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans. Were we to require Phase II testing in order to prove utility, the associated costs would prevent many companies from obtaining patent protection on promising new inventions, thereby eliminating an incentive to pursue, through research and development, potential cures in many crucial areas such as the treatment of cancer." *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995). Thus, the information provided herein is sufficient to overcome the utility rejection.

The examiner also rejects tissue specificity as an adequate utility. On Page 4 of the Office action, it is stated: "The fact that this protein is only expressed in epididymis does not provide a substantial utility since the instant specification does not identify any disease or disorder which can be diagnosed by the detection of the presence or absence of this protein or which can be treated by the addition or removal of this protein."

The examiner provides no scientific reasoning for rejecting the adequacy of the tissue marker utility as disclosed in the application. There is no statutory requirement that a disease or disorder association be necessary to establish a patentable utility. Example 6, beginning on Page 27 of the Patent Office's published "Synopsis of Application of Written Description Guidelines," describes a cDNA fragment of a glial specific G-coupled protein receptor which is "disclosed as useful in drug discovery methods." A claim to the fragment was considered patentable under §112, first paragraph. See, Example 6, Page 29. There was no disease association; only tissue specificity was shown. It would have been disingenuous for the Patent Office to offer this claim and disclosure as an example to the public, if such claim were deficient on other grounds, i.e., §101, especially when §101 was subject of another official PTO publication. Thus, the Patent Office, itself, has not questioned tissue specificity as adequate to fulfill the statutory requirements to get a patent.

Rejection under §112, second paragraph

The term "derivative" is fully described in the specification. See, e.g., Page 9, line 31-Page 10, line 30. Claims have also been added which recite that the derivative has sequence identity to SEQ ID NO:2 (claims 31) and that it hybridizes to SEQ ID NO:2 (claim 32). Hybridization-type claims were described by the Patent Office as having a format in conformance with the §112 requirements. Compare, e.g., Example 9 of the "Synopsis of Application of Written Description Requirement."

It is stated that claims 2, 3, and 5 are indefinite because "they appear to be drawn to both a protein and a fragment, each of which appears to be an alternative embodiment of the other. Clarification and/or alternative wording is requested. As indicated by the claim, the claimed protein is a protein "derivative" of SEQ ID NO: 2 or a protein "fragment" of SEQ ID NO: 2. Thus, it is not understood what is unclear with the claim wording. Clarification and/or a suggestion for alternative wording is requested.

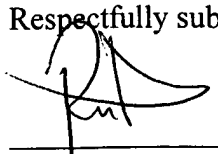
Rejection under §102

This rejection is respectfully traversed. The examiner did not specifically identify any deficiency in 112, first paragraph. Thus, the examiner has failed to establish a prima facie case, and the rejection must be withdrawn.

In view of the above remarks, favorable reconsideration is courteously requested. If there are any remaining issues which could be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,



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Reversible Immunocontraception in Male Monkeys Immunized with Eppin

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Various forms of birth control have been developed for women; however, there are currently few options for men. The development of male contraceptives that are effective, safe, and reversible is desired for family planning throughout the world. We now report contraception of male nonhuman primates (*Macaca radiata*) immunized with Eppin, a testis/epididymis-specific protein. Seven out of nine males (78%) developed high titers to Eppin, and all of these high-titer monkeys were infertile. Five out of seven (71%) high–anti-Eppin titer males recovered fertility when immunization was stopped. This study demonstrates that effective and reversible male immunocontraception is an attainable goal. This method of immunocontraception may be extended to humans.

Although several different choices and approaches are available for contraception in women, the choices for men are currently limited to condoms and vasectomy (*1*, *2*). Male hormonal contraceptives (*3*, *4*) developed over the past several years have now advanced to clinical trials, and the outcome of these studies may determine whether the suppression of sperm production through androgen regulation can become a realistic product. Immunocontraception, an alternative nonhormonal method, has been studied for many years (*1*, *5*), with the major emphasis on immunization of females to prevent pregnancy (*6*) or fertilization (*7*). In the present study, we report the successful contraception of male nonhuman primates (*M. radiata*) immunized with Eppin, a testis/epididymis-specific protein (*8*, *9*). This represents a non–hormonally disruptive male immunocontraceptive for primates.

Before using the monkey as a model for the test of a male immunocontraceptive, we determined the presence of both Eppin and immunoglobulin (IgG) in the male reproductive tract, the immunogenicity of Eppin, and the effects of immunization on sperm motility at the University of California, Davis (UC-Davis). Results from these studies are shown in figs. S1 to S3 and the supporting online material text (*10*). The studies on normal *Macaca* monkeys at UC-Davis allowed us to determine that IgG is present in the normal epididymal tract. Moreover, immunization of two monkeys at UC-Davis indicated that the immune response to recombinant Eppin could influence the titer of antibodies to Eppin in their semen. These results are similar to a previous study in female macaques demonstrating that different serum titers resulted in correspondingly different antibody titers in oviductal fluid (*11*). Consequently, in the fertility study described below, two male monkeys in the initial immune group were dropped because they could not sustain a high serum titer and were unlikely to have a high semen titer. The lack of a strong immune response to an immunogen in a particular individual animal is a reflection of the major histocompatibility complex and T cell response (*12*) as well as the antigen's availability to regulate the immune response (*13*). Such responses are found in heterozygous populations and would need further study before proceeding with additional Eppin fertility trials, which might include a linear B cell epitope (fig. S7).

We tested the effect of Eppin immunization on male fertility at the Indian Institute of Science. Six adult male monkeys (*M. radiata*) were immunized with human recombinant Eppin and six controls received adjuvant only. High–anti-Eppin titers were detected in four of the six monkeys immunized with Eppin in squalene; two monkeys were low responders with titers <1:400 on postimmunization day

Table 1. Fertility test of Eppin-immunized male monkeys (*M. radiata*). The seven males were 7 to 12 years old and had each sired 1 to 3 offspring in the previous 1 to 2 years. No pregnancies resulted from immunized males. Each exposed female had an estradiol 17β surge, indicating that an ovulation probably occurred in that cycle. ELISA O.D., enzyme-linked immunosorbent assay O.D. at 1:1000 on nearest day of cohabitation.

Male monkey no.	Cycle no.	Female monkey no.	ELISA O.D.	Days after first immunization
		Group 1 Eppin (squalene)		
602				
	I	55	0.39	476
	II	88	1.19	522
	III	107	1.19	528
619				
	I	84	0.67	475
	II	64	0.56	523
	III	23	0.56	530
625				
	I	60	1.48	397
	II	97	1.00	525
	III	89	1.15	568
679				
	I	19	0.70	475
	II	47	0.62	534
	III	82	0.62	542
		Group 2 Eppin (CFA)		
610				
	I	29	0.91	147
	II	73	0.59	225
	III	97	1.19	250
656				
	I	107	1.86	147
	II	47	1.93	225
	III	55	2.14	250
657				
	I	79	1.27	147
	II	6	1.53	276
	III	82	1.00	323

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Table 2. Fertility test of male monkeys (*M. radiata*) in adjuvant control group. The six males were 10 to 11 years old and had each sired 1 to 3 offspring in the previous 1 to 3 years. Male 609 impregnated two different females. Four out of six males (67%) impregnated females.

Male monkey no.	Female monkey no.	Days after first immunization when conceived
569	37, 107, 47	No pregnancy
604	35	650
607	87	650
609	86; 6	502; 594
688	96	502
690	6, 84, 11	No pregnancy

126. Because the purpose of this study was to test the efficacy of antibodies to Eppin on fertility, the two low-titer monkeys were dropped from the study group and their fertility was not tested. Three additional males were added, which were immunized (primary immunization) with recombinant human Eppin in complete Freund's adjuvant (CFA) to boost immunogenicity. The original four monkeys immunized with Eppin in squalene were designated group 1, and the three additional males immunized with Eppin in CFA were designated group 2.

Antibody titers in all of the monkeys were >1:10,000 at the time the matings started and remained elevated throughout the mating period. Figure S4, A (group 1) and B (group 2), shows the mean optical density (O.D.) value at 450 nm for the monkeys at a 1:1000 dilution of serum. A titer of >1:1000 was sustained for 775 days in group 1 (fig. S4A) and for 481 days in group 2 (fig. S4B). There was no effect on serum testosterone levels in the immunized males in either group 1 or group 2 compared with control values (fig. S5) and no effect on sperm counts in either group (fig. S6).

Each male monkey in the immune and control groups was subjected to fertility testing by cohabiting with a proven fertile female between days 9 and 14 of her menstrual cycle. Each male was exposed to three ovulatory cycles of three different females to test their fertility. Immune and control groups began fertility testing on days 390 to 397 (Table 1 and table S2; for group 2, day 390 is 147 days after their first day of immunization). Group 1 completed testing on day 568, group 2 completed testing on day 566 (323 days after their first day of immunization), and the control group completed testing on day 691 (table S2). None of the immunized monkeys was able to impregnate females, indicating that males with sustained high–anti-Eppin titers were infertile (Table 1). Four monkeys in the adjuvant control group impregnated 5 females (4 out of 6, 67%, Table 2). All the monkeys used for breeding exhibited ovulatory cycles (table S1).

After the completion of fertility testing, immunizations of monkeys stopped on day 691 (day 448 of immunization for group 2). Groups 1 and 2 were maintained without further immunizations for 450 days (group 1; Eppin/squalene) and 451 days (group 2; Eppin/CFA), respectively, to test their ability to recover fertility after immunization. During this recovery time period, three of four monkeys in the Eppin/squalene group and two of three monkeys in the Eppin/CFA group recovered their fertility for a total recovery of 71% (5 out of 7; table S3). The males exhibited no symptoms of autoimmune disease and had no detectable serum titer of antibody to Eppin at 1:1000 dilutions.

This study demonstrates that effective and reversible male immunocontraception in primates is an attainable goal. We found that a high serum titer (>1:1000), sustained over several months, achieves an effective level of contraception. Seven out of nine males (78%) developed high titers to Eppin, and all these high-titer monkeys were infertile. Five out of seven (71%) high–anti-Eppin titer males recovered fertility when immunization was stopped.

Eppin on the surface of spermatozoa and in semen is bound to semenogelin (*14*), which is involved in coagulum formation in the ejaculate. We can speculate that one mechanism to explain the infertility is that antibodies to Eppin interfere with normal Eppin interaction with the sperm surface and with semenogelin.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/306/5699/1189/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Tables S1 to S3
References

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A Cluster of Metabolic Defects Caused by Mutation in a Mitochondrial tRNA

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Hypertension and dyslipidemia are risk factors for atherosclerosis and occur together more often than expected by chance. Although this clustering suggests shared causation, unifying factors remain unknown. We describe a large kindred with a syndrome including hypertension, hypercholesterolemia, and hypomagnesemia. Each phenotype is transmitted on the maternal lineage with a pattern indicating mitochondrial inheritance. Analysis of the mitochondrial genome of the maternal lineage identified a homoplasmic mutation substituting cytidine for uridine immediately 5′ to the mitochondrial transfer RNA^{Ile} anticodon. Uridine at this position is nearly invariate among transfer RNAs because of its role in stabilizing the anticodon loop. Given the known loss of mitochondrial function with aging, these findings may have implications for the common clustering of these metabolic disorders.

Hypertension and dyslipidemia are important risk factors for many common cardiovascular diseases, including myocardial infarction, stroke, and congestive heart failure (*1*, *2*). These traits are concordant in individual patients more often than expected by chance (*3*, *4*). Large epidemiologic studies have demonstrated that subjects with hypertension

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New approaches for male fertility control: HE6 as an example of a putative target

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Abstract

Reversible contraceptive methods for males are still not available. During the last few years several marketing studies have clearly shown that men and women would welcome a situation where men could assume responsibility for family planning. Schering AG and Organon are currently collaborating to develop a hormonal method for male fertility control based on the combination of etonogestrel as gestagenic component and testosterone undecanoate.

To further optimize male contraceptives in terms of improved efficiency, rapid onset, reversibility, fewer side effects and a convenient method of application, a search for innovative non-hormonal approaches was started. During the last few years, numerous proteins were identified which play a specific role in male fertility. These proteins have first to fulfil a set of indication-specific criteria before a drug discovery process can be initiated. The most important criteria for a putative target protein are tissue-selective expression, crucial biological function in fertility, drugable properties and feasibility of assay development for high-throughput-screening and lead optimization.

The G-protein-coupled receptor HE6 was selected as target and the above selection criteria were applied. HE6 displays a preferred epididymis-specific expression pattern and belongs to the superfamily of GPCRs, which are well known to be drugable with small molecules. A knockout mouse was generated which revealed an infertility phenotype with the onset occurring 6 weeks after initiation of spermatogenesis at the latest. Surprisingly, no epididymis-specific phenotype was observed. Instead, the reabsorption of testicular fluid along the efferent ducts was strongly affected. No further obvious side effects were observed in male or female mice. This study with HE6 exemplifies how targets for male contraception have to be validated before drug development can start.

Keywords: Male contraception; HE6; GPR64; Knockout mouse; Epididymis; Efferent duct; Drug targets

1. Introduction

Although women can choose from a wide variety of contraceptive methods today, no reversible and efficacious methods for men are available. Condoms have the advantage of protecting against sexually transmitted diseases, but are associated with a high failure rate of around 12% (Trussell and Kost, 1987) as it is the case for another contraceptive option for man, coitus interruptus. The third option, vasectomy, cannot be considered as a reversible method since vasovasostomy does not reconstitute fertility in all patients (Pasqualotto et al., 2004). During the last few years, several surveys have clearly shown that the acceptance rate for male fertility control could reach up to 80% depending depending on the methods and population groups (Martin et al., 2000; Heinemann et al., 2005).

As early as 1939, Heckel (1939) and McCullagh and McGurl (1939) showed that testosterone could induce fully reversible azoospermia in men. Exogenous administration of sex hormones suppresses the hypothalamic–pituitary axis. Decreased pulsatile gonadotrophin-releasing hormone (GnRH) secretion from the hypothalamus and reduced production of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) lead to low intratesticular testosterone concentration and to an inhibition of spermatogenesis (Wu, 1996, Nieschlag and Waites, 1996). This principle of the hormonal method of male contraception was further developed and tested in clinical trials using combinations of progestins or GnRH analogous, together with testosterone (Perheentupa and Huhtaniemi, 2004; Wang and Swerdloff, 2004). Several clinical studies have shown that testosterone/progestin combinations suppress spermatogenesis

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to azoospermia without causing severe side effects in close to 90% of men (Amory, 2004).

At Schering AG a search for innovative non-hormonal approaches was initiated to overcome certain drawbacks of male hormonal contraceptives. No less than 2 months administration of the hormonal combinations are necessary for men to reach the low sperm concentrations in their ejaculates that guarantee contraceptive efficacy. Alternative approaches intend to shorten this time of onset and reversibility of contraception. In addition, new methods of male contraceptives should allow a more convenient way of application, enhancing their acceptability. Recent studies with male hormonal contraceptives based on combinations of androgens and progestins used mostly intramuscular testosterone injections at 6–12 weekly intervals (for overview see Wang and Swerdloff, 2004). The progestin part was administered as an injection, implant or daily oral pill. Moreover, a non-hormonal male contraceptive should display high selectivity. In particular, the identification of target proteins, which are specifically expressed in the male reproductive tract may allow the development of highly selectively acting drugs with excellent safety profiles. Since male contraceptives would be taken by healthy men for long periods of time the safety is of the utmost importance.

2. Prerequisites for a target protein from the industrial perspective

At the current time two principal technical approaches are available to interfere with essential functions in male fertility: immunocontraception and a drug-based contraception.

To be accessible for antibodies, protein targets for immunocontraception have to be expressed on cell surfaces. The antigen–antibody interaction should lead to functional impairment, apoptosis or can result in sperm cell aggregations. While several immunocontraceptive approaches have been tested in females, less experimental data are available for males. So far, no research studies have demonstrated high efficacy rates combined with low side effects. In a recent study, O'Rand et al. (2004) immunized bonnet monkeys against human eppin, a sperm-specific protease highly conserved in monkey and men. Seven of nine monkeys developed sufficient antibody titres to ensure contraception. However, only five of seven monkeys regained fertility within the observation time of more than 14 months. Similar results have been obtained in other studies. The main difficulty of immunological approaches is controlling the intensity of immune reaction for each individual. Consequently, the optimal immunization protocol to achieve a sufficient, but not long-term antibody induction is unpredictable. The risk of irreversible infertility caused by inflammation processes within the reproductive tract is unacceptable for human use.

Schering AG has focused on a second approach — male contraception based on small molecules interfering with key fertility functions. Prior to screening, criteria were defined which putative target proteins for a non-hormonal male contraceptive approach should fulfil. A general overview of the target discovery process is provided by Egner et al. (2005). The most important criteria for the screening and evaluation of putative protein targets for male fertility control are summarized below:

1. Biological importance for fertility: the current method of choice to validate the function and importance of a certain target protein for fertility is the generation of transgenic animals (targeted gene disruption, overexpression of dominant negative mutants or in vivo RNAi approaches). Transgenic mouse models are most commonly used for evaluation since, despite considerable effort, no models are available which reproduce all details of the complex processes of spermatogenesis or epididymal sperm maturation in vitro. Moreover, transgenic mouse models allow the identification of unpredicted target protein effects in organs or cells where a phenotype is least expected. Cumulative and age-dependent effects can complete the functional understanding. Despite the power of transgenic technology for in vivo target validation, the application of this technology and in particular gene knockout approaches have drawbacks due to the long development time necessary for the generation and evaluation of such a model. New progress in short-hairpin RNA (shRNA) knockdown technology now allows the generation of knockdown mice in less than 5 months starting with selected shRNAs, and may provide an alternative genetic approach for in vivo target validation.

   Generation and evaluation of a transgenic animal model for proof-of-concept studies is only valid if the protein of interest is sufficiently evolutionary conserved in its function across species boundaries, allowing the transfer of findings in the mouse to the human situation.
2. Expression pattern: using bioinformatic tools, microarray technologies, in situ hybridisation and, if feasible, immunohistochemical methods, the expression profile of the target protein has to be analysed. A highly restricted organ- or cell-specific expression pattern (e.g. expression only within post-meiotic spermatids) is clearly preferred. Side effects of compounds directed against highly specifically expressed protein targets can be confidently attributed to the drug properties.

   If a target validation in the mouse is planned, the expression profile between human and mouse has to be compared in detail. Otherwise, findings and observations from the in vivo model cannot be transferred to the human situation with confidence.
3. Drugability: the preferred drug compound of the pharmaceutical industry is of relative small size (e.g. <500 Da). Small drug-like molecules display improved pharmacokinetic and dynamic properties. In addition, synthesis and large-scale production is more economical. Therefore, target proteins should have small, defined and accessible binding niches; properties most often displayed by proteins with enzymatic or receptor functions. Many proteins possess multiple functional domains. In those cases, it is important to know that the enzymatic function to be inhibited by a small molecule is essential for fertility and not, for example, the scaffold function of the enzyme which cannot be inhibited by small drug compounds. Genetically modified animals carrying muta-

tions in the enzymatic domain leading to enzymatic inactivity, but maintained protein expression can help unravel the multiple functions of a single protein.

Furthermore, male contraceptive drugs should block the target highly selectively. To increase the likelihood of identifying target-selective compounds, the binding niche of the target protein has to be significantly different from that of other members of the protein family or other proteins with similar binding niches. A three-dimensional modelling based on the crystal structures of similar enzymes can help to predict the binding niche properties (Egner et al., 2005).

4. Feasibility: lead compounds for further optimization are routinely identified via the high-throughput-screening (HTS) of large compound libraries. Therefore HTS-compatible, robust in vitro assays have to be developed which provide simple detectable read-outs like phosphorylation or second messenger generation. To develop appropriate assays for a certain protein target, a minimal functional knowledge of the molecular pathways is necessary. Receptor ligands or enzyme substrates are often mandatory for a successful assay development. Sometimes the expression of the target protein is difficult or inefficient. In particular, membrane proteins like GPCRs or ion channels are difficult to express in a functional manner and in sufficient amounts. In addition, biologically relevant in vitro assays have to be established to select and assess compounds during the optimization phase. For drug development in the field of male contraception, assays for sperm motility or acrosomal reaction are available but in vitro assays for spermatogenesis are still under development.

For economical reasons, the patent situation is of great importance and significantly influences the decision of pharmaceutical companies to invest in the development of a certain drug. Data from newly approved pharmaceuticals estimate the average drug development costs of up to US$ 800 million already in 2001 and the time to market of 12 years in average (DiMasi et al., 2003).

3. Processes where drugs can block fertilization

The entire life cycle of a germ cell is a highly complex and precisely regulated process controlled by a wide variety of factors. The identification and characterization of the key factors is the first important step in approaching putative drug targets. The decision on which process and function should be targeted has direct influence on the possible onset and recovery time of a new male contraceptive. Furthermore, the way a drug has to be administered can be already affected by this choice.

The life cycle of germ cells starts with the first mitotic divisions of spermatogonia followed by the meiotic phase and the final differentiation of spermatids to spermatozoa. After completion of spermiogenesis and spermiation, sperm cells are passively transported into the epididymis. During their passage through the epididymal ducts, a poorly understood maturation process takes place, which is a prerequisite for the sperm cell's ability to successfully fertilize oocytes (Cooper, 1995). After ejaculation, spermatozoa have to capacitate in order to achieve the competence for fertilization. Directed motility ensures that the sperm achieve physical proximity with the oocyte–granulosa cell complex. After recognising the zona pellucida, sperm cells have to initiate the acrosomal reaction to penetrate the extracellular matrix surrounding the oocyte. Sperm hyperactivity at this point of the fertilization process is crucial in order to reach the oocyte membrane surface. Finally, the spermatozoan binds to receptors anchored to the oocyte membrane and the membrane fusion process takes place. For most of these processes, essential proteins have been identified during the last few years (Fig. 1). Matzuk and Lamb (2002) collected more than 200 mouse mutations causing reproductive defects. These models support the identification of potential new contraceptive targets. Using the criteria listed above, putative drug targets can be efficiently selected. A few examples of putative pharmacological drug targets are depicted below.

The most advanced approach for a new male contraceptive – the hormonal approach – suppresses sperm production. Mechanisms through which suppression of gonadotrophins and intratesticular testosterone exert their effects on spermatogenesis are not yet completely understood. Studies have shown that a combination of decreased proliferation of type B spermatogonia, increased apoptosis of pachytene spermatocytes and round spermatids, and a disturbed timing of spermiation causes the observed oligo- or even azoospermia (Wang and Swerdloff, 2004).

The whole process of spermatogenesis takes 64 days in human (Clermont, 1972). The interference with a drug at very early stages of spermatogenesis, e.g. proliferation of spermatogonia, would result in a long onset time for the contraceptive effect, an important parameter for the acceptability of a new drug. Furthermore, a direct drug interaction with the stem cell pool of men bears some risk of irreversible effects.

Furthermore, interference with meiotic processes is not preferred due to safety issues. Drug interference during the meiotic phase of chromosome recombination could theoretically lead to genetic alterations. As an example, the completion of meiotic division is impeded in cyclin-dependent kinase 2 (Cdk2) knockout mice, however, no genetically altered sperm have been found in male knockout mice (Berthet et al., 2003). Germ cells of these mice never complete spermiogenesis thus causing an azoospermic phenotype.

Contraceptive approaches blocking post-meiotic functions could in theory lead to controlled infertility within 3 weeks of the first drug application. The post-meiotic phase of human spermatogenesis lasts about 16 days. Of particular interest in this process are kinases, which are often key enzymes in essential cellular pathways and, in addition, well established drug targets. This enzyme family can be inhibited through small molecules that directly block the catalytic activity by interfering with the ATP or substrate binding.

Examples of kinases essentially involved in post-meiotic processes have been reported. CaMK4, a multifunctional serine/threonine protein kinase, is involved in post-meiotic chromatin condensation. Disruption of the Camk4 gene in mice results in male sterility, impairment of spermatogenesis and abnormal sperm head morphology (Wu et al., 2000). Recently,

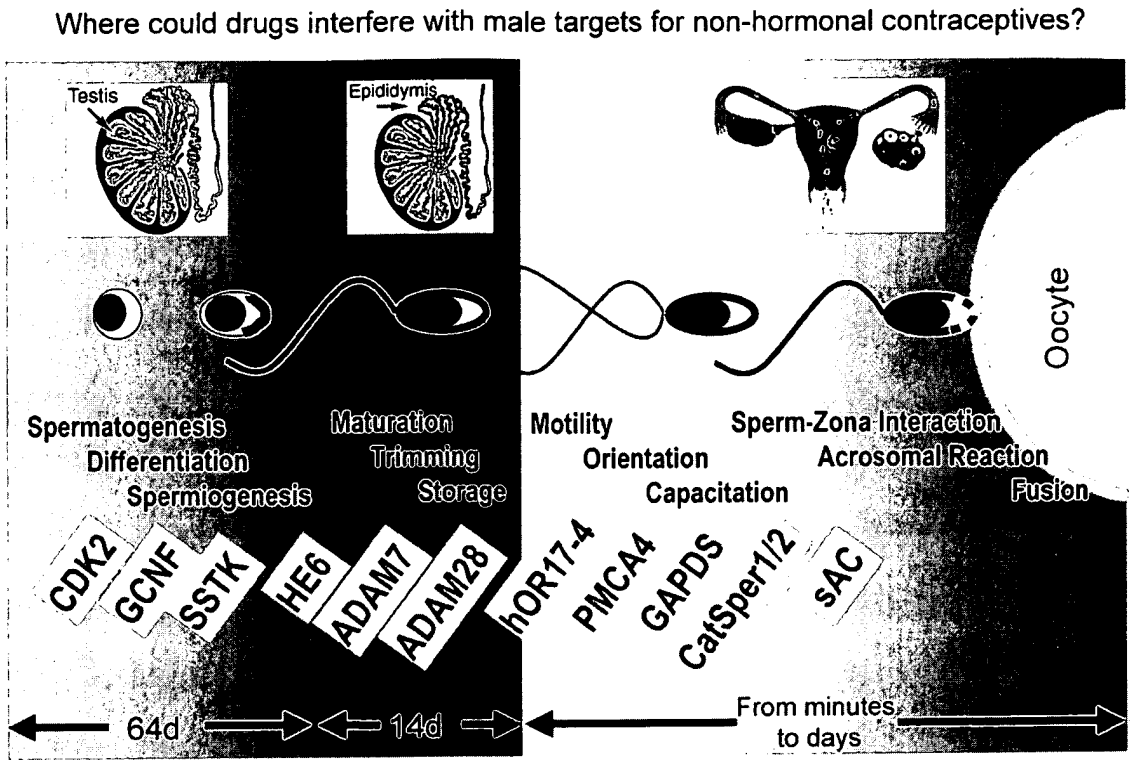

Fig. 1. Overview of putative male-specific drug targets for new approaches of non-hormonal contraception. Targets involved in key functions of spermatogenesis and epididymal maturation could be used to develop male contraceptives. Sperm targets which exert their functions in the female reproductive tract might also be evaluated for innovative female contraceptives. Indicated target proteins are discussed in the text. The time scale at the bottom represents the length of the different events of the sperm life cycle. The time of onset and reversibility of contraception is directly linked to the stage where sperm cell function is disrupted.

similar observations were made in mice with targeted disruption of the small serine/threonine kinase (SSTK) gene (Spiridonov et al., 2005). SSTK is required for proper post-meiotic chromatin remodelling. Null mutants display a defect in elongating spermatids at a step in spermatogenesis coincident with chromatin displacement of histones by transition proteins. Drastically reduced sperm counts combined with decreased percentages of motile sperm results in male mice sterility.

Nuclear receptors represent another drugable target family. For steroid hormone receptors, agonists and antagonists have been identified which bind with IC_{50} values in the nanomolar range. The germ cell nuclear factor (GCNF) represents an exclusively testis-specific nuclear receptor and has been suggested to act as a key regulator of post-meiotic gene expression (Ivell et al., 2004). However, validation of a crucial involvement of GCNF in spermatogenesis is still lacking, since conventionally generated knockout mice were found to be embryonic lethal. Moreover, an endogenous ligand for GCNF is yet to be identified and it is still a matter of debate whether GCNF requires any ligand for functional activity at all.

Leaving the testis, mammalian spermatozoa transit along the excurrent duct system formed by the vasa efferentia, epididymis and vas deferens. Primarily the epididymis appears to be essential for the acquisition of male in vivo fertilization capability. During epididymal transit, the male gamete undergoes major structural, biochemical and physiological modifications. Therefore, contraception based on interference with epididymal function is conceivable. In addition, the onset time to achieve contraception is likely to be further minimized compared to post-meiotic testicular approaches.

The general organization and function of the human epididymis compared to other mammals seem to be sufficiently conserved to allow the transfer of results from transgenic studies to the human situation, although with some restrictions. The mouse epididymal transcriptome has recently been described by Johnston et al. (2005), a study which represents a comprehensive source for segment-specific epididymal genes.

We analysed epididymis-specific expressed disintegrin and metalloproteases containing proteins (ADAMs) as putative drug targets. ADAM7 as well as ADAM28 are predominantly expressed within the epididymis. Especially, ADAM7 displays an optimal tissue expression pattern in mouse and man, being exclusively expressed in epididymal tissues (Gottwald, unpublished data). The metalloproteinase function represents a favourable site where ADAM proteins could be targeted with small molecules. Unfortunately, ADAM7 exhibits an altered zinc binding sequence within its catalytic site that has led to the assumption that ADAM7 exhibits no enzymatically active metalloprotease domain (Lin et al., 2001).

Shortly after ejaculation, sperm cells have to display essential functional characteristics in the female reproductive tract that might lead to additional attractive contraceptive targets. Sperm motility is the most obvious feature that guarantees that sperm

cells reach the oocyte. In addition, hyperactivated movement is a key flagellar function of sperm cells required for egg penetration. Several proteins important for this process have been described which underline the importance of unmodified sperm motility for successful fertilization in vivo. Loss of the sperm cell restricted cation-channel proteins CatSper 1 or CatSper 2 results in motility and hypermotility defects and sterility in male mice (Ren et al., 2001; Quill et al., 2003). Furthermore, sperm motility is severely impaired in the glyceraldehyde 3-phosphate dehydrogenase-*S* (GAPDS) knockout mice (Miki et al., 2004). The human ortholog, GAPD2, is the only GAPDH isozyme in human sperm, making it highly attractive as a contraceptive target. Also, deficiency of the plasma membrane Ca^{2+}-ATPase Isoform 4 (PMCA4) leads to male infertility in mice due to impaired sperm motility (Schuh et al., 2004; Okunade et al., 2004)). All these potential targets belong to well-known drugable protein families.

Data from Spehr et al. (2003) support the hypothesis that human sperm utilise the odorant receptor hOR17-4 for chemotaxis and orientated movement towards the oocyte. Although further experimental data are certainly necessary to fully understand the biological relevance of these findings, odorant receptors belong to the superfamily of G-protein-coupled receptors (GPCRs), which represents the most important class of target proteins for drug discovery (Wise et al., 2002).

Finally, the process of capacitation is necessary for sperm cells to obtain the ability for acrosomal reaction after zona pellucida binding. The acrosomal reaction is a crucial exocytotic event that sperm undergo to be able to penetrate the extracellular envelope of the oocyte. The bicarbonate-dependent soluble adenylyl cyclase (sAC) has been suggested to be essential in initiating sperm capacitation. In sperm of sAC-deficient mice, forward motility was completely lacking (Esposito et al., 2004). Motility in these sperm could however be restored by the addition of external cyclic AMP (cAMP). cAMP therefore seems to be the essential second messenger triggering the final sperm capacitation process occurring in the female reproductive tract. Thus, the inactivation of mouse adenylyl cyclase 3, the only other adenylyl cyclase expressed in spermatozoa, also results in a male infertility phenotype (Livera et al., 2005), emphasising the importance of a tight regulation of cAMP as a key factor for motility, capacitation and the acrosomal reaction.

Pharmaceutical interference with sperm proteins involved in motility, late events of sperm maturation or egg recognition and binding, offers the possibility of developing these drugs as male and/or female contraceptives. Irrespective of their activities, all sperm proteins are already expressed in late spermatids before leaving the testis. Pharmaceutical drugs applied in the man could already bind to those proteins in the male reproductive system and be in place to prevent target activation within the female reproductive tract. On the other hand, inhibiting male target proteins in the female reproductive tract may offer the advantage of being very safe and may also be effective when used only precoitally. A new generation of sperm-targeted female contraceptives can be envisaged which can be administered "on demand."

4. Assessment of HE6 as epididymal target for male fertility control

Pharmaceutical interference with crucial functions of the epididymis might have the advantage of shortening the time to achieve infertility, an important parameter influencing acceptability of the marketed drug. Therefore, we searched for attractive epididymal targets which fulfil the above specified target criteria as completely as possible.

Osterhoff et al. (1997) described the cloning of HE6, also referred to as GPR64, a new epididymis-specific orphan GPCR, identified by differential screening of a human epididymal cDNA library. The sequence of HE6 reveals the presence of a GPCR-typical highly conserved seven transmembrane (TM7) domain and a C-terminus, containing all structural prerequisites for G-protein coupling. However, the most striking feature of HE6 is the large ectodomain reminiscent of a receptor subfamily that has been classified as LNB-TM7, belonging to the family B of GPCRs. Homology searches within the TM7 domain have shown some similarities with the secretin/vasointestinal peptide superfamily of GPCRs.

Western blot analysis demonstrated that HE6 is cleaved into a two-subunit proteins, comprising of an approximately 180 kDa highly glycosylated ectosubunit and a <40 kDa hydrophobic endosubunit (Obermann et al., 2003). Endoproteolytic processing into two subunits has been shown for other members of the LNB-TM7 family as well, best investigated for the calcium-independent alpha-latrotoxin receptor (Krasnoperov et al., 1997). Both subdomains of HE6 remain membrane associated; the ectodomain could not be detected in epididymal fluids of rodents or on the surface of ejaculated human spermatozoa (Gottwald, unpublished data).

The target criteria, listed at the beginning for potential male contraceptives have been applied to HE6. First of all, an in-depth analysis of the expression pattern of HE6 was performed. Extensive quantitative RT-PCR studies with numerous tissue probes from mouse, rat and human as well as microarray analyses (Affymetrix chips) of essentially all human tissues and organs revealed a highly epididymis-restricted expression of HE6 (Fig. 2). Using HE6-directed antibodies in Western blot analyses and immunohistochemistry, the epididymis-specific expression pattern was confirmed in all species examined. Detailed immunohistochemistry analyses revealed an intense staining of the epithelia of efferent ducts, the stereocilia of the initial segment and the caput region of the epididymis. The overall expression pattern at the mRNA and protein level supported HE6 as a potential fertility target.

All sequence characteristics clearly indicate that HE6 belongs to the GPCR superfamily. For no other class of proteins have more pharmaceutical drugs been developed. Of the approximately 500 currently marketed drugs, more than 30% are modulators of GPCR function. Sequence and structure-based drugability assessment of HE6 enforces the assumption that small drug-like molecules modulating the function of HE6 could be identified.

To develop screening assays for the identification of small molecule ligands that either activate or block receptor func-

even assuming that such disclosure is lacking, knowledge of mechanism is at most only relevant where the disclosed utility is not believable. See, e.g., *Newman v. Quigg*, 877 F.2d 1575, 11 USPQ2d 1340 (Fed. Cir. 1989) having claims to a perpetual motion machine (i.e., energy output is greater than energy required to operate the machine); *In re Dash and Keefe* (Fed. Cir. 2004; no-precedential) having claims to a cold fusion machine. The examiner has not explained why it is inherently incredible to believe, e.g., that ligands to HE6, such as antibodies, as disclosed in the pending application would act as contraceptives in the manner disclosed in the specification.

Although unnecessary, since the examiner has failed to establish a *prima facie* case of lack of utility, attached is a publication (Exhibit 1) by Gottwald et al. Dr. Gottwald is an employee of the assignee of the present application and previously provided a Declaration under §1.132. This article describes the efforts to identify male contraceptive agents. Evidence is provided of the specificity of HE6 (as disclosed in the pending application) and its importance in male fertility. For example, knockout mouse were almost sterile. See, Paragraph spanning Pages 6-7 of Gottwald et al. See, also previously filed Declaration by Gottwald under §1.132. On Pages 2-3 of the publication, the authors describe different criteria for identifying targets for male contraception agents. HE6 is evaluated by these criteria, and the authors conclude (e.g., on Page 8, second column) that it is a valid target for contraception purposes. Thus, the stated contraception utility in the application is clearly credible to the person of ordinary skill in the art, the standard set forth in the Patent Office's Guidelines. See, MPEP §2107. II. Examination Guidelines for the Utility Requirement.

It is not necessary to provide evidence that a particular compound (e.g., an antibody to HE6) was used in a therapeutic context. As discussed in *In re Brana*, this is not the correct standard to apply. "Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans. Were we to require Phase II testing in order to prove utility, the associated costs would

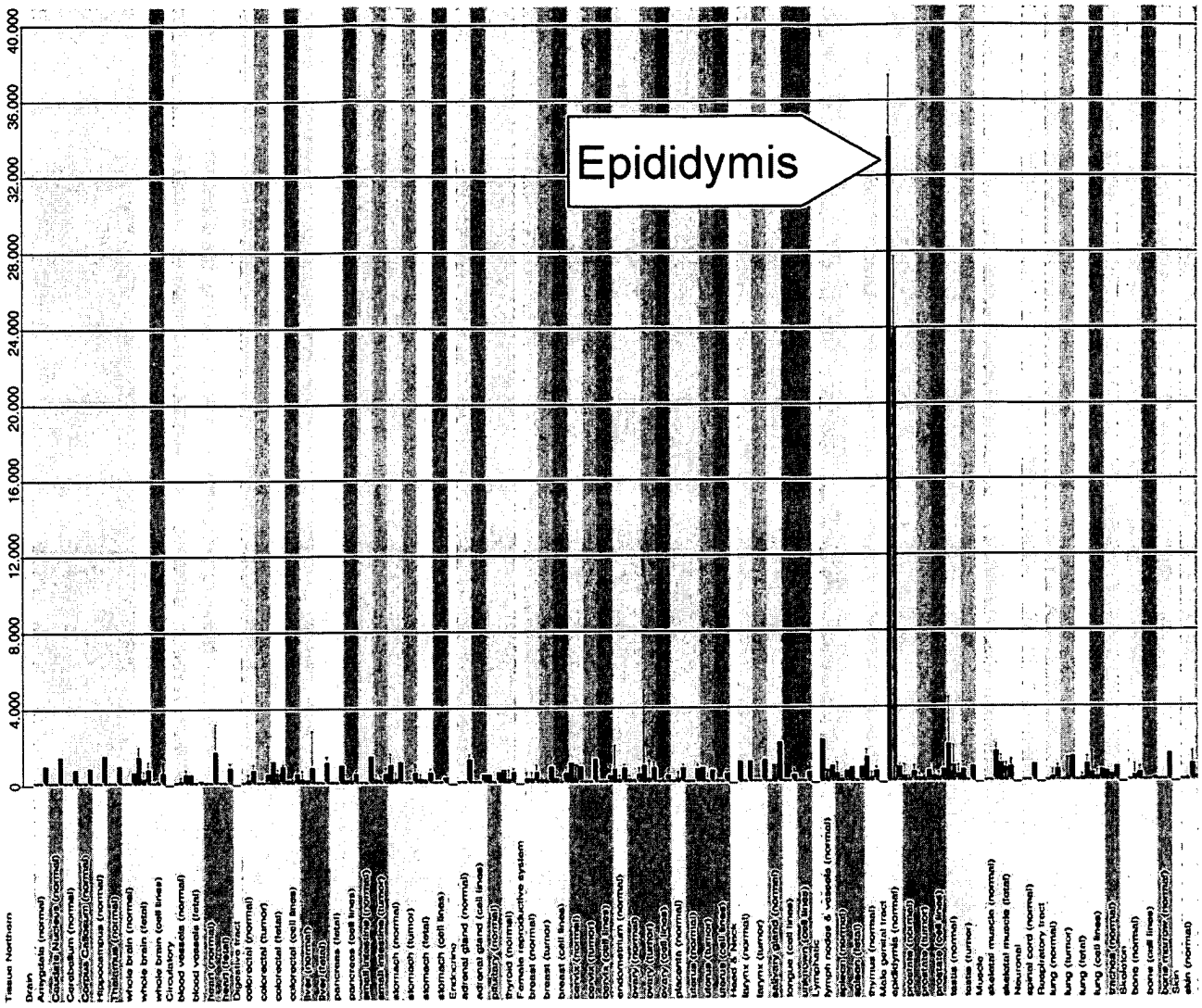

Fig. 2. Expression profile of HE6 using Affymetrix chip analyses. The gene expression values for HE6 of several human tissue samples are displayed as a bar graph on an arbitrary scale. Overall more than 600 tissues samples (adult, fetal and tumour) and cell lines were analysed for HE6 expression. HE6 is represented by three different hybridisation probe sets. For details see Egner et al. (2005).

tion, a basic knowledge of ligand and pathway information is of major importance. HE6 was grouped by phylogenetic analysis to the adhesion family of GPCRs (Fredriksson et al., 2003). However, a ligand prediction for GPCRs is impossible since ligands for GPCRs are associated with tremendous variation. Despite several attempts to isolate an endogenous ligand for HE6, e.g. using testicular or epididymal fluids, no active fraction could be detected so far. Foord et al. (2002) noted that HE6 activates G-protein Gs/Gq when overexpressed in *Xenopus* melanophores.

The Gs-coupled signal pathway activates the cellular adenylyl cyclases causing increased cAMP levels. Therefore, HE6 activity might be monitored by measuring the changes of cAMP levels. To overcome the missing ligand for HE6 two strategies are appropriate. Knowing the measurable read-out of activated HE6, agonists might be identified by screening compound libraries. In case of success, the identified HE6-selective agonist can then be used in a competition assays to screen for potent antagonists. Another option might be to overexpress HE6 in a way that a ligand-independent constitutive activity can be observed (Chen et al., 2000; Parnot et al., 2002). Constitutive activity would allow screening for antagonists as well as for inverse agonists. In short, screening orphan GPCRs for ligands has to be considered as challenging.

Finally, the biological importance of HE6 for male fertility has to be evaluated. Therefore, we generated a knockout mouse model and investigated the resulting phenotype in detail (Davies et al., 2004). In knockout mice, the lack of HE6 protein expression was confirmed by Western blot analyses and immunohistochemistry. HE6 knockout animals displayed no apparent developmental or behavioural abnormalities compared with wild-type littermates. Body and organ weights showed no obvious deviations. Testosterone and gonadotrophin levels were unchanged. Of major interest was the fertility phenotype of the knockout mice. Knockout males at the ages between 6 and 80 weeks were examined in fertility studies. Mating behaviour was unchanged compared with wild-type littermates. However, knockout males at 6–9 weeks of age already displayed a significant subfertility. Knockout males older than 15 weeks were

almost sterile. Of the 15 different knockout males older than 15 weeks which were studied, only one produced a single litter. All fertility studies were performed with NMRI females, a mouse strain well known for high fecundity, to allow an assessment of subtle fertility defects. When C57BL/6 females were used in fertility trials, 12 weeks old male knockouts were already unable to produce any offspring (Gottwald, unpublished data).

Histological and functional studies were initiated to understand the cause of this knockout male infertility. Sections through efferent ducts revealed an accumulation of spermatozoa within the distal part close to the initial segment (Fig. 3). An observed expanded *rete testis* of knockout mice pointed to a back pressure of retained testicular fluids. However, animals at the age of 2 weeks already displayed expanded *rete testis*. Mice at this age have not yet begun spermatogenesis, so that the accumulation of sperm cells has to be interpreted as a secondary effect. All findings in HE6 knockout males pointed to a defect in reabsorption of testicular fluids, taking place primarily within the efferent ducts. To further support these findings, we microdissected efferent ducts from wild-type and knockout mice and cultured ligated pieces for up to 96 h similar to the studies by Hess et al. (1997). Using this ex vivo model, we compared the function of the ductules from wild-type and knockout littermates. Whereas ligated ductules of wild-type mice were capable of removing the luminal fluid as measured by unchanged or decreased diameters over time, ductules from knockout animals showed significantly increased diameters, once again suggesting that the loss of HE6 function results in defects in luminal fluid reabsorption (Fig. 4).

To assure that even in mice with increased age no complete atrophy of the germ cell epithelia occurred due to fluid accumulation, mice up to the age of 18 months were analysed. The early stages of spermatogenesis were always present, supporting the suggestion that reversibility of spermatogenesis would be possible in principle.

5. Outlook

Multiple surveys have clearly demonstrated a demand for male contraceptives (Heinemann et al., 2005). Although the hormonal approach of male contraceptives is far advanced, several aspects are expected to be improved by a non-hormonal male contraceptive. First of all, the time of onset and reversibility of sterility can be shortened. The identification of key proteins

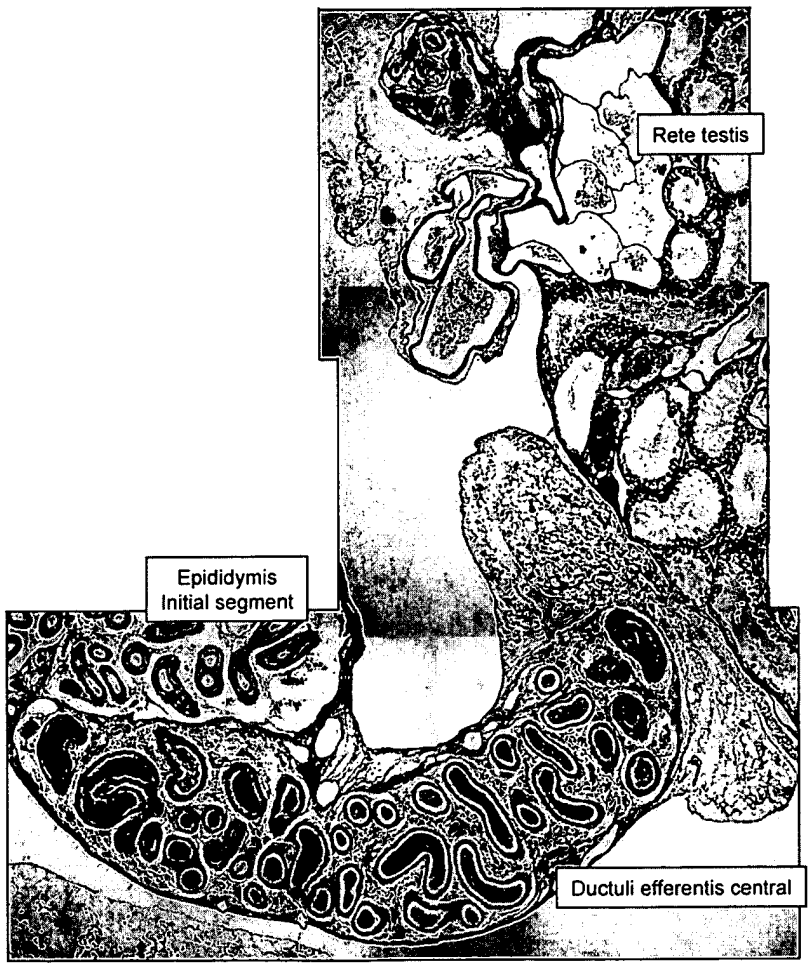

Fig. 3. HE6 knockout phenotype. In 8-week-old mice spermatozoa have already accumulated. However, the initial segment as well as all other parts of the epididymis are free of sperm cells. An expanded rete testis is clearly visible, indicating an increased fluid pressure. Seminiferous tubules are found to be dilated with reduced spermatogenesis in many tubules.

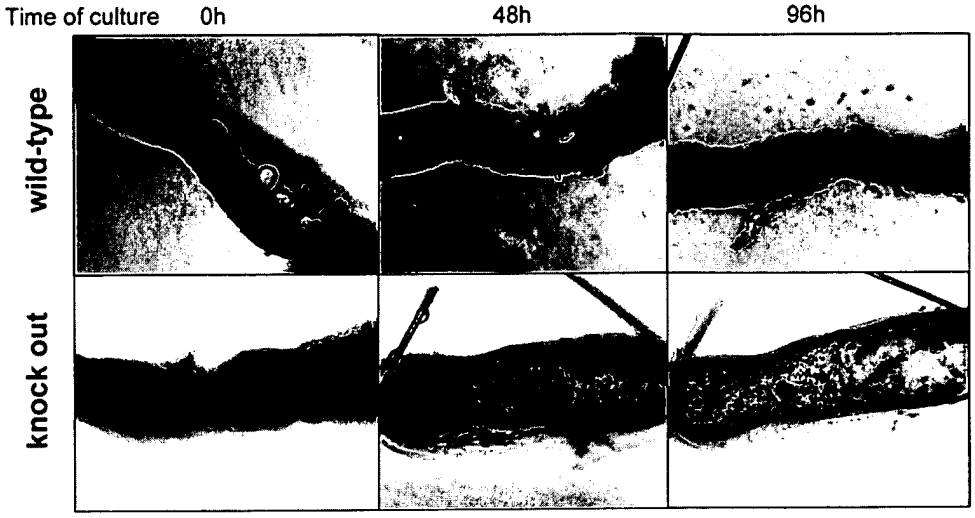

Fig. 4. Ex vivo culture of ligated efferent ductules. Ductulus pieces were ligated, microdissected and cultured for up to 96 h similar to the study described by Hess et al. (1997). Rapid ciliary beat was used as marker for cell integrity. The luminal ductule diameter of wild-type mice appeared unchanged over several days, whereas the lumen of HE6 knockout mice was always significantly increased, indicating a disturbance in fluid reabsorption.

for post-meiotic differentiation, epididymal maturation, sperm motility, capacitation or acrosomal reaction would allow the long onset and reversibility times to be overcome. Taking into account that healthy men would take a male contraceptive for long periods, only drugs displaying virtually no side effects are to be developed. Therefore, target proteins have to be chosen, which are preferentially expressed only in cells of the male reproductive tract, thus minimizing undesired drug side effects. Potential drug compounds must interfere with high selectivity and superior efficacy. In addition, the mode of the drug application influences its acceptability for men enormously.

When starting to search for optimal drug targets, it is extremely difficult to foresee all potential adverse effects, which may be later observed in men. Although hundreds of male-specific genes are already identified, our understanding of male reproductive physiology and the underlying molecular pathways is still at the beginning. To deepen our knowledge in male reproduction with special emphasis on post-testicular processes, in 1997 the Rockefeller Foundation together with the Ernst Schering Research Foundation initiated a research network of 10 top-level research institutions called Application of Molecular Pharmacology for Post-testicular Activity (AMPPA) (Diczfalusy et al., 2004). The great scientific success of the AMPPA network led to a continuation of this program in 2003. AMPPA2, now supported by CONRAD, focuses on the selection and validation of potential drug targets for a male contraceptive. To our knowledge the AMPPA program was the first ever where a humanitarian foundation and a foundation of a commercial company came together on a global scale to create a research network. The new venture achieved sustained success as public–private partnership, as collaborative scientific program and as an approach to identify new drug targets, underlining the importance of public–private partnership for this purpose.

To optimize the target selection process, we have developed a set of criteria that putative drug targets should preferably fulfil. Here we have applied these criteria to HE6. The assessment revealed that drugability, the favourable expression pattern and the knockout phenotype support the selection of HE6 for the development of a drug antagonist. However, the screening of orphan receptors such as HE6 directly for antagonists is often not feasible. In many cases, ligand identification is a first mandatory step in the screening process. Beside HE6, several other male-specific key factors are already described, increasing the possibility that a drug for non-hormonal male contraception could be developed in the near future.

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